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A comparative study on solvent extraction effect on the phytochemical profiling and antioxidant capacity of *Zizyphus lotus* L and *Glycyrrhizaglabra* L roots.

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Abstract

This research work aims to determine the total phenols, flavonoids, condensed tannin levels and antioxidant power of methanolic and aqueous extracts of *Zizyphus lotus* L and *Glycyrrhizaglabra* L roots, using three methods (Test DPPH, bleaching of β-carotene and hemoglobin denaturation protection). The higher levels of total phenols were found in methanol extracts of *G. glabra* and *Z. lotus* (58.94 ± 0.05 and 51.80 ± 0.02 mg GAE/ g of extract, respectively), the methanolic extract of *Z. lotus* was the richest in flavonoids and tannins condensed with a contents of 16.00 ± 0.01 and 17.22 ± 0.1 mg CE/g of extract respectively. With the DPPH method, the water extract of *Z. lotus* represents significantly ($p < 0.05$) the most extract active (04.52 ± 0.17 mg / ml). For bleaching of *β*-carotene, *G. glabra* aqueous extract showed significantly ($P < 0.05$) a higher antioxidant activity (71.47±01.86%). The methanol extracts of *G. glabra* and *Z. lotus* showed inhibition of hemoglobin denaturation with values of 71.80±0.06 and 71.71±0.33%, respectively. Based on these results, it could be concluded that the *G. glabra* and *Z. lotus* roots are a rich source of natural antioxidants of high value.

Keywords: Flavonoids, Phenolics, *Zizyphus lotus* L, *Glycyrrhizaglabra*L, Free Radicals, Antioxidant activity.

Introduction

Cells and tissues can be subject to a wide assortment of physical, substance and metabolic assaults. Most of these lead to a common expression know as “oxidative stress”, due to the exaggerated production of free radicals derived from oxygen a physiological phenomenon that, is generally well controlled (Walker et al., 1982). Oxidative pressure is characterized as an irregularity between the age of receptive oxygen species and the body's capacity to neutralize and fix oxidative harm (Boyd et al., 2003; Tang & Tsao, 2017; Mertoğlu et al., 2020). Free radicals are mainly produced by domestic sources, for example, electron transport chains, peroxisomes and the cytochrome P-450 framework. They are responsible for the alteration of DNA and the cellular aging that underlies certain diseases, such as cancer (Favier, 2003). Natural molecules capable of delaying or preventing oxidation are thus an interesting potential source, as plants extracts contain a variety of phenolic compounds and essential oils with antioxidant capacities.

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Glycyrrhizaglabra L. is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China. Pharmacological studies have confirmed that plant extracts and individual biologically active compounds exhibit a broad range of biological activitiessuch as hypocholesterolemic and hypoglycemic (Sitohy, El-Massry et al., 1991), antimicrobial (Anagha et al., 2014), antiviral (Cinatl et al., 2003), antiallergic (Kroes et al., 1997), anti-ulcer (Da Nagao et al., 1996), antidiabetic (Isbrucker & Burdock, 2006), antioxidant (Belinky et al., 1998), anti-inflammatory (Fujisawa et al., 2000), and hepatoprotective activities (Wu, Shen, Yin, Yu, & Meng, 2006).

*Zizyphus Lotus*L. *(Z. Lotus)*, also known as jujube, belongs to the Rhamnaceaefamily. This family includes about 135–170 species of Zizyphus(Maraghni, Gorai, & Neffati, 2010). In Africa, *Z. Lotus* is widely distributed in Mediterranean region, like Algeria, Morocco, Libya and Tunisia. This plant is employed in nutrition, health, and cosmetics in several forms. In traditional medicine, both in North Africa and Middle East, several parts of *Z. lotus* are given ashypoglycemic, and gastroprotective actions (Benammar et al., 2014), antiurinary troubles agents, skin infections, antifever, antidiarrheal activities (Anand et al., 1989) and antimicrobial activity (Rais et al., 2019).

The point of this work was to evaluate the absolute phenols, flavonoids and consolidated tannin of *Glycyrrhizaglabra* and *Zizyphus lotus* L. roots extracts. The assurance of the antioxidant movement of the concentrates was assessed utilizing three unique techniques by comparing the effect of extraction solvent on the antioxidant activity of extracts.

Material and Methods

Plant material

The *Glycyrrhizaglabra* roots were collected in December 2015 from El-karimia region of Chlef (Latitude: 36 ° 06'42,80 "N, Longitude: 1 ° 33'10, 17 "E, Altitude = 218 m) Algeria. The *Zizyphus lotus* L roots were harvested in November 2015 from the same location (Latitude: 36 ° 07'32 "N, Longitude: 1 ° 32'33.71" E, Altitude = 210m)Algeria. After drying at room temperature, the plant materials were crushed into a powder using an electric mortar.

Preparation of root extracts of *G. glabra* and *Z. lotus*

Preparation of aqueous extracts

The aqueous extracts were prepared by macerating 30 g of root powder from each plant in 300 ml of distilled water. After 72 hours, the extracts were, filtered followed by concentration using a rotary evaporator at 40°C. Drying was carried out in an oven heated to 40°C.

Preparation of methanolic extracts

To prepare the methanolic extracts, 30 g of powder from each plant was mixed with 300 ml of a methanolic solution for 72 hours. After filtration, the extracts were concentrated using a rotary evaporator (48°C) and then dried in an oven at 40°C (Cheurfa & Allem, 2016).

Evaluation of total phenols content

The assurance of total phenols was done spectrophotometrically utilizing Folin-Ciocalteu reagent strategy (Wong et al., 2006). 0.5 ml of each concentrate was added to 2.5 ml of the Folin Ciocalteu solution (weakened multiple times in distilled water), and 2 ml of the 7.5% sodium carbonate solution; the absorbance is estimated at 760 nm after 30 min of incubation in the dark. The phenol content in the concentrates was determined from a setup calibration curve with the exact amount of gallic acid. The outcomes were represented in milligrams of gallic acid identical per gram of powdered concentrate (mg GA E/g).

Determination of total flavonoids

The total flavonoid content was determined spectrophotometrically utilizing the aluminum chloride method.

1.0 ml of each extract was added to 5 ml of distilled water and 0.3 ml of $NaNO_2$ (5%). After 5 min, 0.6 ml of an ethanol solution of $AlCl_3$ (10%) was added. 2.0 ml of a solution of sodium hydroxide (1.0M) was added after 5 min, then the volume was completed to 10 ml with distilled water, then, the absorbance was determined at 510 nm (Zhishen, Mengcheng, & Jianming, 1999). The total flavonoid content of the extracts was expressed in milligrams of quercetin equivalent per gram of plant powder (mg QE /g).

Determination of condensed tannin

The condensed tannin was analyzed following the method reported by (Sun et al., 1998). Where 50 µl of each extract was added to 3 ml of a methanolic solution of vanillin (4%), followed by the addition of 1.5 ml of concentrated HCL. The mixture was incubated for 15 minutes. The absorbance was measured at 500 nm against the methanol. The condensed tannin content results were expressed in milligrams of catechin equivalent per gram of plant powder (mg Cq / g).

Antioxidant activity using Di-Phenyl-Picryl-Hydrazine (DPPH) test

The DPPH is a violet stable free radical in solution, it exhibits a characteristic absorbance in 517 nm, this color disappears rapidly when the DPPH is reduced in Di-Phenyl-Picryl-Hydrazine by a compound with antiradical property (Sanchez-Moreno, 2002). 1.0 ml of methanolic solution of DPPH (0.004%) was added to 1 ml of different concentrations of each extract where a standard antioxidant (ascorbic acid), the mixture was vigorously stirred, then incubated at room temperature and in the dark. After 30 minutes, the absorbance was reported at 517 nm. The IC50 was determined for each extract, it is defined as the concentration that gives 50% inhibition of DPPH (Hu et al., 2018).

Bleaching of β-carotene test

(Tepe et al., 2004) method was utilized to study the antioxidant activity of the extracts. A solution of *β*-carotene in chloroform was added to 25 µl of linoleic acid and 200 mg of Tween 40. After evaporation of the chloroform, 100 ml of the water saturated with oxygen were added, with vigorous stirring until the formation of an emulsion. In test tubes, 2.5 ml of the emulsion was added to 350 µl of each extract (200 mg/ml) followed by incubation in the dark at room temperature. Two control tubes were likewise arranged with a similar strategy: one containing a reference butylatedhydroxytoluene (BHT) (positive control) and the

other a negative control where the example is supplanted by 350 µl of methanol. Absorbance was estimated at 490 nm following 48 hours.

Protection of hemoglobin against denaturation

The antioxidant activity of the extracts was determined according to the method of (Fant et al., 2005). Where 0.1 ml of the various extracts was mixed with 0.025 ml of linoleic acid (0.1 M) in an ethanol solution, 0.075 ml of phosphate buffer (0.2 M, pH 7.2), and 0.05 ml of hemoglobin (0.08%). The reaction mixtures were incubated at 37°C for one hour, then the reaction was stopped with the addition of 5 ml of a solution of HCl (0.6%) in ethanol. Then 0.02 ml of a solution of $FeCl_2$ (0.02 M) and 0.01 ml of ammonium thiocyanate (30%) were added to 1.6 ml of the sample solution. Ascorbic acid and gallic acid were utilizd as a positive control. Absorbance was estimated at 490 nm.

Statistical analysis

The information introduced in this investigation was examined utilizing XL Stat Pro 7.5 statistical software. The tests were done in triplicate. Results were introduced as mean and standard deviation. . One-way ANOVA procedure was used for multiple comparison at a P< 0.05 significance level.

Results

Total phenols content

The total phenol content of the various extracts is shown in Table -1-. Total phenol contents of the methanolic extracts was higher than those found with the aqueous extracts (P <0.05). The methanolic extract of *G. glabra* has significantly (P <0.05) a higher total phenol content (58.94 ± 0.05 mg GAE/g extract), followed by the methanolic extract of *Z. lotus* (51.80 ± 0.02 mg GAE/g extract) (Table 1). These levels were significantly (P <0.05) higher than those found with the aqueous extracts of *Z. lotus* and *G. glabra* whose contents were 25.88 ± 0.03 and 07.57 ± 0.01 mg GAE/g of xtract, respectively (Table 1). Total phenol contents ranging between 09.68 ± 1.01 and 56.7 ± 05.93 mg GAE/g extract were found by (Akram et al., 2015) with methanolic, ether and chloroformic extracts of *G. glabra*.(Bakhtaoui et al., 2014) study on the methanolic extract of *Z. lotus* showed that the total phenol content was 1200 µg CAE/g.

Total flavonoids

The flavonoid contents are shown in Table -1-. The flavonoid contents of the *Z. lotus* extracts were significantly (p <0.05) higher than the levels found with *G. glabra* extracts. The quantitative analysis of flavonoids showed that the methanolic extract of *Z. lotus* is significantly (p <0.05) the richest extract of flavonoids with a content of 16.00 ± 0.01 mg QE / g of extract. A non-significant difference (p> 0.05) was observed between the aqueous extract of *Z. lotus* and the methanolic extract of *G. glabra* whose contents are of 09.96 ± 0.02 and 08.31 ± 0.65 mg QE/g of extract respectively. The low content (p <0.05) was recorded with the aqueous extract of *G. glabra* (05.77 ± 0.02 mg QE/g extract).

The study conducted by (Suhayla, 2014) showed that the flavonoid content of the aqueous and methanolicextracts of *G. glabra*were found 2.3% and 6.8% respectively.

Condensed tannin

The condensed tannin assay shows that their content in the methanolic extract of *Z. lotus* (17.22 ± 0.1 mg CE / g) was significantly (p <0.05) higher than the other extracts (Table 1). A condensed tannin content of 3.32 ± 0.1 mg CE/g was foundin the aqueous extract of *G. glabra* roots followed by the aqueous extract of *Z. lotus* (2.94 ± 0.1 mg CE/g). In contrast, the methanolic extract of *G. glabra* showed a lower level (1.64 ± 0.1 mg CE/g) (Table 1).

Table 1. Total phenols, flavonoids and tannin Content of *G. glabra and Z. lotus* extracts

Extracts	Total phenols (mg GAE/ g)	Flavonoids (mg QE/g)	Tannin (mg CE/g)
*G. glabra*aqueous extract	7.57 ±0.01^{d}	5.77 ± 0.02^{d}	3.32 ±0.1^{b}
G. glabra methanolic extract	58.94 ±0.05^{a}	8.31 ± 0.65^{c}	1.64 ±0.1^{d}
Z. lotus aqueous extract	25.88 ± 0.03^{c}	9.96 ± 0.02^{b}	2.94 ±0.1^{c}
Z. lotus methanolic extract	51.80 ± 0.02^{b}	16.00 ± 0.01^{a}	17.22 ± 0.1^{a}

Values with the same lowercase letter are not significantly different (*P>0.05*).

DPPH test

The antioxidant activity of the various extracts of *G. glabra* and *Z. lotus* against the DPPH radical was evaluated. Among the tested four extracts, the aqueous extract of *Z. lotus* represents significantly (p <0.05) the most antioxidant activity with IC_{50} of 04.52 ± 0.17 mg /ml (Table 2). In contrast, the aqueous extract of *G. glabra* was the least active with a value of 52.87 ± 0.57 mg/ ml (Table 2). The methanolic extract of *G. glabra* also recorded a significant antioxidant effect (p <0.05) with IC_{50} of 10.02 ± 0.56 mg/ ml. The IC_{50} of the methanolic extract of *Z. lotus* was 60.56 ± 0.70 mg /ml. Ascorbic acid showed IC_{50} of 1.93±0.55 mg/ml, which was significantly lower (P <0.05) than the IC_{50} of all extracts tested (Table 2). Sultana et al., (2010) studied the antioxidant activity of the methanolic extract of *G. glabra*plant, they recorded an IC_{50} value of 87.152 µg/ml. (Naz et al., 2013) studied the antioxidant activity of the leaves of the five *Zizyphus*genus, a content of 71.9 ± 0.17% was found with the methanolic extract of *Z. lotus*.

Bleaching of β-carotene test

The aqueous extracts showed significantly (p<0.05) higher percentages of antioxidant power than the methanolic extracts. The aqueous extract of *G. glabra* showed significantly higher antioxidant power compared to the other extracts tested (71.47 ± 01.86%). The antioxidant power of the aqueous extract of *Z. lotus*and methanolic extract of *G. glabra* were67.80 ± 0.59 and 64.20 ± 0.51%, respectively(p>0.05) (Table 2).

The antioxidant power of these extracts was greater than the antioxidant power of the *Z. lotus* methanolic extract (15.77 ± 0.25%) (p <0.05) (Table 2). On the other hand, these values

are significantly lower than BHT antioxidant power (93.98 ± 0.42%) (Table 2).

The results showed that the oxidation of linoleic acid was effectively inhibited by the extracts tested. A more pronounced effect (EC_{50} = 23 µg/ml) was observed by (Gormez & Sengul, 2007) for ethanolic extracts of licorice roots collected in Turkey (EC_{50} = 75 µg/ml). The results reported by these authors ranged from 28.03% (25 µg / ml) to 88.07% (800 µg / ml).

Hemoglobin protection against denaturation

The inhibition peroxidation percentages (Table 2), revealed that the root extracts of *G. glabra* and *Z. lotus* displayed a significant protective effect of hemoglobin vis-à-vis the peroxide radical. The methanolic extracts displayed a protective activity significantly greater than that of the aqueous extracts of *G. glabra* and *Z. lotus* ($p < 0.05$). A non-significant difference ($p > 0.05$) was seen with the methanolic extract of *G. glabra* and *Z. lotus* (71.80 ± 0.06% and 71.71 ± 0.33% respectively). The protective power of the *G. glabra* aqueous extract was 60.50 ± 0.13% : a non-significant difference ($p > 0.05$) with gallic acid (61.15 ± 0.13%) and the aqueous extract of *Z. lotus* (59.60 ± 0.19%). These results were significantly higher ($p < 0.05$) compared to ascorbic acid (53.14 ± 0.19%) (Table 2).

The results indicated that the methanolic extracts of *G. glabra* and *Z. lotus*, which have a high content of total phenols and flavonoids, display the best protective activity for hemoglobin.

Table 2. Results of the antioxidant activity of the extracts tested.

Tests **Extracts**	**DPPH Test (IC_{50} mg/ml)**	**Bleaching of β-carotene test (%)**	**Hemoglobin protection (%)**
G. glabra aqueous extract	52.870 ± 0.57^{d}	71.47 ± 1.86^{b}	$60.40 \pm 0.13^{b,c}$
G. glabra methanolic extract	10.02 ± 0.56^{c}	$64.20 \pm 0.59^{b,c}$	71.80 ± 0.06^{a}
Z. lotus aqueous extract	4.52 ± 0.17^{b}	$67.80 \pm 0.59^{b,c}$	59.07 ± 0.19^{c}
Z. lotus methanoli cextract	60.56 ± 0.70^{e}	15.77 ± 0.25^{d}	71.71 ± 0.33^{a}
AscorbicAcid	1.93 ± 0.55^{a}	-	53.14 ± 0.19^{b}
BHT	-	93.98 ± 0.42^{a}	-
Gallic Acid	-	-	61.15 ± 0.13^{b}

Values with the same lowercase letter are not significantly different (*P>0,05*).

Discussion

The variable phenolic compound contents in these plant species were probably due to a range of factors including the extraction method and the solvent used (El-Waziry, 2007), the biotic (species, organ and physiological stage) and abiotic (edaphic factors) conditions (Ksouri et al., 2008), the nature of the soil, the type of microclimate (Atmani et al., 2009), and the bioclimatic stages in which the plants were grown.

The literature suggested that the antioxidant activity of plant extracts linked on the type of extraction solvent and its polarity with the distribution of antioxidant-active substances between depending on the polarity of the extracts (Kang et al., 2003).

Results obtained for the antioxidant activity of *G. glabra* and *Z. lotus* roots extracts, indicated that their reduced potential is due to the presence of molecules capable of adding electrons, which can react with free radicals and convert them into stable elements, thereby terminating chain reactions involving polyphenols (Ferreria et al., 2008). Phenolic compounds contained in the plants, are endowed with an important antioxidant power, and it has been entrenched that antioxidant activity has emphatically corresponded with the structure of the polyphenols. By and large, polyphenols with a high number of hydroxyl groups have the most elevated antioxidant activity (Heim et al., 2002) because of their capacity to select more atoms to settling free radicals, which may mostly clarify the low cell antioxidant activity of tyrosol, which has just a single hydroxyl group (- OH) in its structure. In this manner, the antioxidant power was portion - needy as well as structure subordinate (De Quirós et al., 2009). Condensed and hydrolysable tannins have also displayed high antioxidant potential in various antioxidant tests (Bors et al., 2001). According to (Sroka, 2005), tannins have a strong antioxidant property due to the high number of hydroxyl groups linked to the aromatic ring.

Conclusion

The high levels of quantitative determination of total phenols (58.94 ± 0.05 mg Eq GA / g), flavonoids (16.00 ± 0.01 mg EQ/g) were found with *G. glabra*methanolic extract.

The study of the scavenging effect of the DPPH radical revealed that the aqueous extract of *Z. lotus* represented significantly the most active extract.

The study of the anti-lipoperoxidant effect of extracts by β carotene bleaching method showed that *G. glabra* aqueous extract was the most active as oxidation inhibitor. The highest percentage of protection of hemoglobin against denaturation was found with the methanolic extract of *G. glabra*.

The antioxidant activity found in *G. glabra* and *Z. lotus* roots extracts gives these plants therapeutic properties. However, further studies *in vitro* and *in vivo* are needed to characterize the potential bioactive compounds.

Conflict of interest

The authors declare that there is no conflict of interests.

Author contributions

K. Zabel and W. Achouni performed the extraction and chemical characterization. M. Cheurfa, K. Zabel and W. Achouni performed the biological experiments. M. Cheurfa and A. Mariod wrote the manuscript. M. Cheurfa, R. Allem and A. Mariod analyzed the data. All the authors revised the manuscript for publication.

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